PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
B01D 57/02

A1

(11) International Publication Number: WO 93/00986

(43) International Publication Date: 21 January 1993 (21.01.93)

(21) International Application Number: PCT/GB92/01238

(22) International Filing Date: 8 July 1992 (08.07.92)

(30) Priority data:
9115073.0 12 July 1991 (12.07.91) GB

(71) Applicant *(for all designated States except US)*: ASTROMED LIMITED [GB/GB]; Innovation Centre, Cambridge Science Park, Milton Road, Cambridge CB4 4GS (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : MACKAY, Craig, Douglas [GB/GB]; 53 Hamilton Road, Cambridge CB4 1BP (GB). BARKER, Richard, Frederick [GB/GB]; 86 Hollytrees, Bar Hill, Cambridge CB3 8SG (GB).

(74) Agent: KEITH W. NASH & CO.; Pearl Assurance House, 90-92 Regent Street, Cambridge CB2 1DP (GB).

(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: A METHOD AND AN APPARATUS FOR ELECTROPHORETIC SEPARATION

(57) Abstract

A method and apparatus for electrophoretic gel imaging in which two transparent plates are secured together to define between them at least one track for electrophoretic gel which has a thickness or depth in the range 25 to 250 microns, typically of the order of 50 microns, (i.e. in the range 25 to 100 microns). In an embodiment the two plates define a plurality of parallel tracks each of the order of 200 microns width, achieved by grooving one of the plates with rectangular cross-sectioned slots. A reduced gel running time of 20 to 30 minutes is possible, compared to the normal 3 to 5 hours.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CS	Czechoslovakia
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
PL	Poland
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

A METHOD AND AN APPARATUS FOR ELECTROPHORETIC SEPARATION

Field of the Invention

This invention relates to a method of and apparatus for electrophoretic separation which have particular, but not exclusive, application in DNA sequencing.

Background to the Invention

DNA sequencing procedures used currently generally take a run of several hours in order to give a useful sequence run-out of several hundred base-pairs. This applies as much to conventional radio-labelled electrophoretic slab gel sequencing procedures as it does to the latest automated DNA sequencers which use fluorescently labelled primer, such as those marketed by Applied Biosystems and Millipore. The time is required to move the DNA fragments through a gel medium such as polyacrylamide or agar far enough to give the necessary resolution. The resolution required is that needed to produce the n th band as being separated from the n+1 th band. Clearly the width of a band must be less than this separation if the bands are to be distinguished reliably. The characteristic width of a band is generally limited to the thickness of the gel for a variety of reasons, so in practice gels of thickness of 0.5-1.5 mm are frequently used, necessitating the use of very long gels in order to achieve the resolution

necessary to work with a maximum value of n that can be excess of 1000.

A recent proposal to speed up DNA sequence analysis is known as capillary gel electrophoresis. However, this proposal has several practical limitations, one of which is the deleterious effect that increased electric field strength can have upon resolution.

The present invention is concerned, inter alia, with the problem of realising a scaled down DNA sequencing system, especially but not exclusively a system employing fluoresecent gel imaging. In practice, the imaging aspects of such a scaled down system are not a problem; the difficulties arise in miniaturising the sequencing gel system and in loading the gel with scaled down volumes.

The Invention

According to one aspect of the present invention, there is provided a method of electrophoretic separation in which an electrophoretic gel is run between two transparent plates which are secured together to define a gel thickness in the range 25 to 250 microns.

More especially a gel thickness of the order of 50 microns is preferred, where the term "of the order of 50 microns" is used herein and in the appended claims to indicate a gel thickness generally in the range 25 to 100 microns.

Reducing the gel thickness from the usual thickness of 0.5 to 1.5 mm substantially reduces the gel running time necessary to achieve a given resolution, because the reduced thickness enables finer bands to be achieved with

a given imaging system, thus enabling the required resolution to be achieved with a shorter than normal gel. In practice, the length of the gel may be as short as about 60 mm. The term "about 60 mm" is used herein and in the appended claims to indicate a gel length generally in the range 40 to 100 mm.

For convenience, the width of gel tracks is also reduced to a small multiple of the gel thickness, say between 2 and 10 times the gel thickness. Thus, in practice, a typical gel track width of about 200 mm may be employed. The term "about 200 mm" is used herein and in the appended claims to indicate a width generally in the range 100 to 500 mm.

With a reduced gel track width, it is possible to run several tracks of electrophoretic gel between the two transparent plates.

Thus, according to another aspect of the present invention, there is provided a method of electrophoretic separation in which an electrophoretic gel is run in parallel tracks defined between two transparent plates one of which is grooved with slots of rectangular cross-section to define the track widths, track depths and track spacings.

According to still another aspect of the invention, there is provided apparatus for electrophoretic separation which comprises two transparent plates secured together to define parallel tracks between them for receiving and running an electrophoretic gel, one plate being flat and the other being grooved with slots of rectangular cross-section to define the track widths, track depths and track

spacings.

The invention is applicable to electrophoretic separation (one-dimensional) of a wide range of materials, including proteins, carbohydrates and DNA fragments, and finds particular application in DNA sequencing.

It is possible to consider in a more detailed manner how the various parameters operate if the scale of a DNA sequencing system is scaled by a multiplication factor X, where X is less than 1 and preferably X is less than 0.1.

Take a gel of length L, width w, and thickness t. It is run at a mean voltage V for a time T with current I. The electric field inside the gel is V/L and the power dissipated is VI/Lt per unit gel area.

Taking also the case of fluorescent gel imaging, an area A of the gel is illuminated with blue light. This is optically demagnified by a factor m to an area A_D which is the area of a cooled CCD (charge coupled device) detector or other detector used to visualise the fluorescent bands in the gel.

Scaling down the sequencing system by X has substantial beneficial effects on the overall speed and efficiency of the procedure. The resolution is largely although not completely retained if the gel thickness is reduced in proportion to the length. In practice a somewhat larger gel is needed to give the best possible resolution. The electric field needed to move the bands across the gel may be left unchanged, speeding the running of the gel X times. The power dissipation in the gel will be reduced because the current will now be I.X per unit width of the

gel and the power dissipation will also be multiplied by X. It is therefore possible to run the gel in X times the time if the electric field (volts/m) is maintained, or in X^2 times the time if the voltage V is maintained. With a 10-fold reduction in size, a 100-fold reduction in running time could in theory be achieved.

Turning now to the imaging of the gel, a scale factor of X means that the illuminating light intensity will be changed by $1/X^2$, giving a corresponding change in fluorescent output from each fluorescent DNA fragment. Since the demagnification needed is also scaled by X, the efficiency of light transfer is improved by $1/X^2$ giving an overall improvement in the detection signal of $1/X^4$. Unfortunately the situation is not as favourable as this, as the gel loading has also to be reduced. Current gels are already loaded near to their maximum capacity. Overloading causes the bands to be broadened, degrading resolution. To maintain the concentration of fluorescent fragments in the gel the loading has to be changed by X^3, giving a net sensitivity improvement of only 1/X.

In summary therefore scaling a DNA fluorescent sequencing system by multiplying all scales by X allows a running time to be achieved between X and X^2 times that previously taken and a detection sensitivity that scales as 1/X. This gives an overall efficiency that scales to $1/X^3$. In practice the gains achieved are less than this but they are still very substantial.

The following explanation is relevant to the case of a DNA sequencing system using fluorescent gel imaging, but the stated figures should be considered only to indicate the order of magnitude of dimensions involved and should not

be considered limitative.

Starting with a CCD, assumed to be 1000 pixels across the gel each of 20 microns, the gel then has an active width of 20 mm assuming 1:1 imaging from the gel to the CCD, which is the most efficient arrangement for light transfer. The gel may have 80 tracks, each 250 microns apart. The tracks may be 200 microns wide and have 50 micron gaps between them.

As the CCD has 20 micron pixels, band widths need not be any smaller than 40 microns, since the CCD will limit resolution to about this level. A gel thickness of 50 microns will be necessary to keep the resolution of the gel high and minimise the length. A length of 60 mm would allow satisfactory performance. Compared with a current 60 cm gel, this gel can be run at one tenth of the voltage (200 V rather than 2000 V), and a current of only a few milliamps giving a very low cost, much safer power supply, and sequencing can be completed in 20-30 minutes rather than 3-5 hours.

Description of Embodiment

Experiments have shown that the following is the best practical realisation of the invention, for use in DNA sequencing, based on the preceding explanation.

The apparatus comprises two plates approximately 20 mm x 60 mm of suitable material, eg. glass, pyrex (trade mark) or suitable transparent, low fluorescent plastics sheet material. One plate is flat and the other plate is grooved with slots of rectangular cross-section to define the track positions and spacings. The grooves may be

moulded, etched or machined in the plate. At the top of the grooved plate the grooves are made progressively deeper to provide a one-sided funnel to assist with gel loading. These grooves should be several mm deep at the top edge, reducing in depth to the prescribed 50 micron depth a few mm from the top of the gel. The plate includes 80 parallel grooves, each 200 microns wide with 50 micron gaps between them.

The two plates, one flat and the other grooved are clamped together (no spacers are used) and a suitable electrophoretic gel is cast in the usual way. DNA fragments are put into a solution that is loaded into the top of each track using a micro-pipette or micro-syringe, possibly with robotic control. The DNA is forced onto the top of the gel either by a pre-electrophoresis running stage or by centrifugation of the gel plate sandwich (the gel plates are small enough for this to be practicable). The gel is then run in the usual way, but using a voltage of 200V and current of only a few milliamps. The bottom 10 mm, say, of the gel is illuminated by the edge illumination of the gel plates and the emitted fluorescence is transferred by coupling lenses to the cooled, slow-scan CCD detector, as in current practice.

The bands are imaged and the data reduced essentially as in current practice except that the now much better defined gel geometry allows the location of each track to be established much more rapidly and reliably.

Using such apparatus, gels can be run in 20-30 minutes.

While the invention has been exemplified with reference to two transparent plates one of which is grooved to reduce

track widths, it will be understood that the track width is not a significant factor in reducing the gel running time. It is the reduction in gel thickness which is important in achieving a shorter running time.

It is therefore possible to practice the invention with two flat transparent plates which define a reduced gel thickness, as hereinbefore defined and explained. Thus, the particular value of the grooves in one of the transparent plates is to constrain the location of the tracks in which the gel runs. However, it is not essential to constrain the tracks in this way.

Claims

1. A method of electrophoretic separation in which an electrophoretic gel is run between two transparent plates which are secured together to define a gel thickness in the range 25 to 250 microns.

2. A method according to claim 1, in which the gel thickness is of the order of 50 microns (as hereinbefore defined).

3. Apparatus for electrophoretic separation comprising two transparent plates secured together to define between them at least one track for an electrophoretic gel of thickness in the range 25 to 250 microns.

4. Apparatus according to claim 3, in which the plates define between them a gel thickness of the order of 50 microns (as hereinbefore defined).

5. A method of electrophoretic separation in which an electrophoretic gel is run in parallel tracks defined between two transparent plates one of which is grooved with slots of rectangular cross-section to define the track widths, track depths and track spacings.

6. Apparatus for electrophoretic separation which comprises two transparent plates secured together to define parallel tracks between them for receiving and running an electrophoretic gel, one plate being flat and the other being grooved with slots of rectangular cross-section to define the track widths, track depths and track spacings.

7. A method or apparatus according to any of claims 1 to 6, applied to DNA sequencing.

8. A method or apparatus according to claim 7, applied to DNA sequencing using fluorescent gel imaging.

9. A method or apparatus according to any of claims 1 to 8, in which the gel is run between two transparent plates, one of which is grooved with slots of rectangular cross-section to define between the plates a plurality of spaced parallel tracks each having a depth of the order of 50 microns (as hereinbefore defined).

10. A method or apparatus according to claim 9, in which the tracks are each of about 200 microns width and are of about 60 mm length (both as hereinbefore defined).

11. A method or apparatus according to any of claims 1 to 10, in which the gel is run at a voltage of, the order of 200 V.

12. A method or apparatus according to any of claims 1 to 11, in which a CCD is employed for image detection.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01238

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: B 01 D 57/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	B 01 D; G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	DE, A1, 2944127 (C. DESAGA NACHF. ERICH FECHT GMBH & CO) 22 May 1980, see page 17, line 14 - line 39	1-12
A	US, A, 4790919 (CHARLES BAYLOR, JR.) 13 December 1988, see column 4, line 53 - line 55	1-12
A	DETECTION AND QUANTIFICATION OF DNA IN ELECTROPHORESIS GELS AND BLOTS, 1991, vol. 4, VCH, Weinheim, Douglas M. Gersten et al, see page 57 - page 61	1-12

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21st September 1992	19 OCT 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Inger Löfgren

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 92/01238

SA 61625

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/08/92 The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-A1- 2944127	22/05/80	SE-B-C-	452199	16/11/87
		SE-A-	7811720	14/05/80
		US-A-	4337131	29/06/82
		US-A-	4374723	22/02/83
US-A- 4790919	13/12/88	CA-A-	1261784	26/09/89
		DE-A-	3584482	28/11/91
		EP-A-B-	0169397	29/01/86
		JP-A-	61028512	08/02/86
		US-A-	4863647	05/09/89